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Differential expression of leukemia inhibitory factor and insulin like growth factor-1 between normal pregnancies, partial hydatidiform moles and complete hydatidiform moles

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ABSTRACT

Introduction: Leukemia inhibitory factor (LIF) and insulin like growth factor-1 (IGF-1) are two of the most important growth factors mediating trophoblast actions. We hypothesized that the localization and expression patterns of LIF and IGF-1 in partial and complete hydatidiform moles (HM) compared with normal first trimester placentas may provide an understanding of the proliferative processes in HMs.

Methods: The study population included curettage material of women diagnosed as complete or partial HM as a result of histopathological and immunohistochemical examination (complete HM group, n = 8; partial HM group, n = 8) and women undergoing dilatation&curettage for unwanted pregnancies (control group, n = 8). Expression of LIF and IGF-1 among placental cell groups was evaluated immunohistochemically and given a score depending on immunostaining intensity.

Results: In normal chorionic villi strong expression of LIF and IGF-1 was present. Both LIF and IGF-1 expressions were weaker in the chorionic villi of complete HMs. In complete mole decidua there was a significant decrease in glandular and endothelial IGF-1 expression along with a decrease in decidual cell LIF expression compared to normal first trimester decidua. LIF expression in extravillous trophoblasts was stronger in complete molar placentas compared to normal placentas.

Discussion: LIF and IGF-1 are important regulators of trophoblast proliferation and invasion. Differential expression of LIF and IGF-1 in molar trophoblasts and chorionic villi might have a role in regulation of trophoblasts in complete moles. Decreased expression of glandular IGF-1 and decidual LIF might be related to the decidual changes during trophoblastic proliferation and invasion of decidua in complete HMs.

1. Introduction

Hydatidiform moles (HM) are abnormal pregnancies occurring as a result of aberrant fertilization and characterized by proliferation of trophoblastic cells. HMs may be complete (CHM) or partial hydatidiform moles (PHM) and are differentiated by their karyotype and histologic appearance [1].

CHMs are mainly sporadic, diploid and most commonly have a 46,XX karyotype, with all chromosomes of paternal origin [2,3]. The chorionic villi of CHMs are diffusely hydropic and abnormal trophoblastic proliferation involves entire placenta. However in early complete moles hydropic villi may not be apparent and molar stroma may be vascular [4]. On the other hand, PHMs are classically triploid conceptuses due to fertilization of a normal ovum by dispermy so they have both maternal and paternal set of chromosomes [1,5]. Degree of trophoblast proliferation is less and hydropic changes are focal. Embryonic development, fetal vessels and amniotic sac can be found in PHMs.

There is an uncontrolled trophoblastic proliferation in the case of HMs, in contrast to limited invasion and proliferation of trophoblasts in physiological conditions. Decidua, the specialized endometrium of pregnancy, regulates proliferation and invasion of trophoblasts [6,7]. An interaction between decidua and invading trophoblasts limits invasion by producing appropriate cytokines and growth factors [7]. Leukemia inhibitory factor (LIF) and insulin like growth factor-1 (IGF-1) are two of the most important growth factors mediating trophoblast

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actions [8,9].

LIF has an important role in preimplantation blastocyst-endometrium interactions, implantation and early placental development. Additionally LIF induces trophoblastic proliferation and invasion [8]. Furthermore, LIF was reported to promote proliferation and invasiveness of choriocarcinoma cell lines [10]. It is possible that LIF may be playing a role in aberrant trophoblast proliferation in molar pregnancies. To our knowledge, LIF expression in HM has not been studied previously.

IGF-1 is present in almost all cell types of placenta starting from early gestation [9]. IGF-1 is known to positively influence trophoblast migration and invasion. IGF-I may also rescue trophoblasts from apoptosis [11–13]. Furthermore, there are data to implicate IGF-1 in regulating differentiation of cytotrophoblasts into syncytiotrophoblast and extravillous trophoblasts (EVT) [14,15]. Briefly IGF-1 promotes trophoblast survival, differentiation and invasion however it is not clear whether IGF-1 plays a role in HM where uncontrolled trophoblast proliferation takes place.

We hypothesized that the localization and expression patterns of LIF and IGF-1 in PHMs and CHMs compared with normal first trimester placentas may provide an understanding of the invasive and proliferative processes in HMs. Moreover, this finding may help development of diagnostic tools for distinguishing HMs with a greater risk of progression to gestational trophoblastic neoplasia and establishment of new treatment modalities.

2. Methods

2.1. Study subjects

This study was approved by the ethical committee of Hacettepe University Faculty of Medicine, Ankara, Turkey and all samples were used with the informed consents of the patients.

Pathology specimens of 24 women whose uterine curettage, histopathological examinations and diagnosis were performed in Hacettepe University Faculty of Medicine, Ankara, Turkey were enrolled in this study. The study population included curettage material of women diagnosed as CHM or PHM as a result of histopathological and immunohistochemical (targeting p57KIP2 nuclear protein; product of an imprinted gene known to be maternally expressed [16]) examination (CHM group, n = 8; PHM group, n = 8) and women undergoing dilatation & curettage for unwanted pregnancies before 10th gestational week (control group, n = 8). The inclusion criteria included having no p57 immunoreactivity for CHM group and having positive p57 immunoreactivity for PHM group. Curettage materials of women with viable pregnancies who had no abnormal findings in routine examinations were selected for control group. Tissue samples with no determinate diagnosis as CHM or PHM after histopathological and immunohistochemical examinations were excluded. The general characteristics of control, PHM and CHM groups are presented in Table 1.

Table 1
Clinical characteristics of patients with normal and molar pregnancies.

Case Number	Age	Fetal heart beat	Gestational week	P57 Status	Diagnosis	Progression to GTN
C1	41	+	7	Not assessed	NP	–
C2	38	+	7	Not assessed	NP	–
C3	32	+	8	Not assessed	NP	–
C4	28	+	10	Not assessed	NP	–
C5	29	+	6	Not assessed	NP	–
C6	29	+	10	Not assessed	NP	–
C7	22	+	9	Not assessed	NP	–
C8	29	+	9	Not assessed	NP	–
PHM1	29	–	8	+	PHM	–
PHM 2	31	+	16	+	PHM	–
PHM 3	31	–	8	+	PHM	–
PHM 4	33	+	12	+	PHM	–
PHM 5	23	+	10	+	PHM	–
PHM 6	24	+	10	+	PHM	–
PHM 7	32	–	12	+	PHM	–
PHM 8	28	–	10	+	PHM	–
CHM1	25	No embryo	10	–	CHM	–
CHM2	49	No embryo	6	–	CHM	+
CHM3	43	No embryo	8	–	CHM	–
CHM4	41	No embryo	8	–	CHM	+
CHM5	34	No embryo	9	–	CHM	–
CHM6	42	No embryo	8	–	CHM	–
CHM7	36	No embryo	7	–	CHM	–
CHM8	19	No embryo	10	–	CHM	–

Samples are indicated by their group and numbers. *C* control group, *NP* normal pregnancy, *GTN* gestational trophoblastic neoplasia.

2.2. Immunohistochemical staining

Indirect avidin-biotin-peroxidase complex (ABC) method was used to detect LIF and IGF-1 immunoreactivity. Deparaffinated, rehydrated sections were incubated with 0.3% H_2O_2 to block endogenous peroxidase activity; antigen retrieval was performed by boiling the sections in Tris-EDTA buffer (Dr. Zeydanlı, No: DZTAE10- 1). The sections were rinsed in PBS, blocked with 10% normal goat serum, and then incubated with rabbit anti-human LIF polyclonal antibody (1:200, P15018; Abbiotec) and rabbit anti-human IGF-1 polyclonal antibody (1:100, MBS551027; MyBiosource). After washing in PBS, the slides were incubated with a biotinylated secondary antibody (biotinylated goat anti-rabbit IgG secondary antibody). The slides were rinsed once more in PBS and incubated with ABC complex (Vectastain Elite ABC immunoperoxidase detection system, Vector Laboratories Inc., Burlingame, CA, USA) according to the manufacturer's instructions. Counterstaining was done with 10% Mayer's Hematoxylin then washed in cold water and mounted with glycerol-gelatine.

The negative control staining was performed by omitting the primary antibody step and using PBS instead.

2.3. Imaging analysis

Expression of LIF, IGF-1 among placental cell groups (glandular epithelium of endometrium, decidual cells, endothelial cells, EVTs and chorionic villi) were evaluated immunohistochemically and given a score as previously described by Tsai et al. [17]. A Leica DM6000B light microscope was utilized for immunohistochemical evaluation and sections were photographed using a Leica DC500 digital camera. Slides were examined by two independent histopathologists and scored depending on the percentage of immunostaining intensity of the cell type: 0 = no staining; 1 = 1%–30%; 2 = 31%–60%; 3 = 61%–100% (Table 2).

Table 2
Immunoreactivity scores.

Score	Intensity
0	No staining (no immunoreactiviy)
1	Faint staining
2	Moderate staining
3	Strong staining

Table 3
LIF expression among placental cell types in control, PHM and CHM groups.

			Groups		
			Control Number (%)	**PHM** Number (%)	**CHM** Number (%)
LIF	Glandular Epithelium	Moderate staining	0 (0.0)	1 (14.3)	4 (50.0)
		Strong staining	8 (100.0)	6 (85.7)	4 (50.0)
	Decidual Cells [p2,3]	Faint staining	0 (0.0)	3 (37.5)	0 (0.0)
		Moderate staining	0 (0.0)	1 (12.5)	8 (100.0)
		Strong staining	8 (100.0)	4 (50.0)	0 (0.0)
	Endothelial Cells	Faint staining	0 (0.0)	1 (12.5)	0 (0.0)
		Moderate staining	8 (100.0)	7 (87.5)	8 (100.0)
	EVT [p2]	Faint staining	3 (37.5)	1 (12.5)	0 (0.0)
		Moderate staining	5 (62.5)	2 (25.0)	2 (25.0)
		Strong staining	0 (0.0)	5 (62.5)	6 (75.0)
	VT [p2,3]	Moderate staining	0 (0.0)	0 (0.0)	8 (100.0)
		Strong staining	7 (100.0)	8 (100.0)	0 (0.0)

LIF immunoreactivity in placental cell types are scored according to immunostaining intensities: 1%–30% = faint staining; 31%–60% = moderate staining; 61%–100% = ; strong staining. In seven cases of control group, chorionic villi could be analyzed and in seven cases of PHM, endometrial glands could be analyzed. [P] Fischer's exact $P < 0.016$, [1]control vs. PHM, [2]control vs. CHM, [3]PHM vs. CHM group.

2.4. Statistical analysis

All statistical analyses were performed using the MedCalc Statistical Software version 12.7.7 (MedCalc Software bvba, Ostend, Belgium; http://www.medcalc.org; 2013). The immunohistochemistry results were compared between groups using the chi-square test and Fisher's exact test when the expected values were less than 5. For comparison of three groups, a P value less than 0.05 was considered statistically significant. If a significant result was obtained from multiple comparisons, post-hoc analysis was performed. Bonferroni correction was used as the post-hoc multiple comparison procedure. The significance cut off was set at 0.05/3 = 0.016.

3. Results

LIF and IGF-1 expressions among placental cell types were identified immunohistochemically and immunostaining scores were compared between control, PHM and CHM groups (Tables 3 and 4).

In normal first-trimester placentas, LIF immunoreactivity was observed in stromal cells, endothelial lining of decidual blood vessels, EVTs and the chorionic villi (Fig. 1, A, D, G). LIF staining was also present in glandular epithelia and decidual cells. Glandular epithelia showed stronger LIF immunoreactivity than decidual cells. Strong cytoplasmic LIF immunostaining was observed in endometrial glands both in normal placentas and PHMs (Fig. 1, A and E). A similar pattern of strong glandular LIF immunoreactivity was present in CHMs (Fig. 1F). LIF was similarly expressed in endometrial glands of normal and molar placentas (p = 0.05).

In normal placentas, decidual cells showed strong LIF immunostaining (Fig. 1D). The immunostaining intensity decreased in PHMs and CHMs (Fig. 1E and F). LIF staining in decidual cells of CHMs was globally less intense than in normal placentas. However more detailed examinations showed that staining intensity of decidual cells in complete moles differed from each other. Some decidual cells showed stronger immunoreactivity while some stained only in a weak manner (Fig. 1F). Decidual LIF expression was significantly different in CHMs in comparison to both normal first trimester placentas ($p < 0.016$) and PHMs ($p < 0.016$). However there was no statistical difference between control and PHM groups.

In all three groups, endothelial cells lining decidual blood vessels stained positive for LIF whereas smooth muscle cells in vessel wall did not (Fig. 1 A, B, C). In one tissue section of CHM group, myometrial tissue was detected and interestingly myometrial smooth muscles stained positive for LIF (Fig. 1C). This finding seems difficult to interpret because we could observe it in only one sample.

The EVTs of normal placentas showed weak LIF immunoreactivity

Table 4
IGF-1 expression among placental cell types in control, PHM and CHM groups.

			Groups		
			Control Number (%)	**PHM** Number (%)	**CHM** Number (%)
IGF-1	Glandular Epithelium[p2]	Faint staining	0 (0.0)	3 (42.9)	8 (100.0)
		Moderate staining	7 (87.5)	4 (57.1)	0 (0.0)
		Strong staining	1 (12.5)	0 (0.0)	0 (0.0)
	Decidual Cells	Faint staining	8 (100.0)	3 (37.5)	8 (100.0)
		Moderate staining	0 (0.0)	5 (62.5)	0 (0.0)
	Endothelial Cells[p2]	No staining	0 (0.0)	0 (0.0)	1 (12.5)
		Faint staining	0 (0.0)	5 (62.5)	7 (87.5)
		Moderate staining	8 (100.0)	3 (37.5)	0 (0.0)
	EVT	Faint staining	8 (100.0)	3 (37.5)	8 (100.0)
		Strong staining	0 (0.0)	5 (62.5)	0 (0.0)
	VT[p1,2]	Faint staining	0 (0.0)	3 (37.5)	7 (87.5)
		Moderate staining	0 (0.0)	5 (62.5)	1 (12.5)
		Strong staining	7 (100.0)	0 (0.0)	0 (0.0)

IGF-1 immunoreactivity in placental cell types are scored according to immunostaining intensities: 1%–30% = faint staining; 31%–60% = moderate staining; 61%–100% = ; strong staining. In seven cases of control group, chorionic villi could be analyzed and in seven cases of PHM, endometrial glands could be analyzed. [P] Fischer's exact $P < 0.016$, [1]control vs. PHM, [2]control vs. CHM, [3]PHM vs. CHM group.

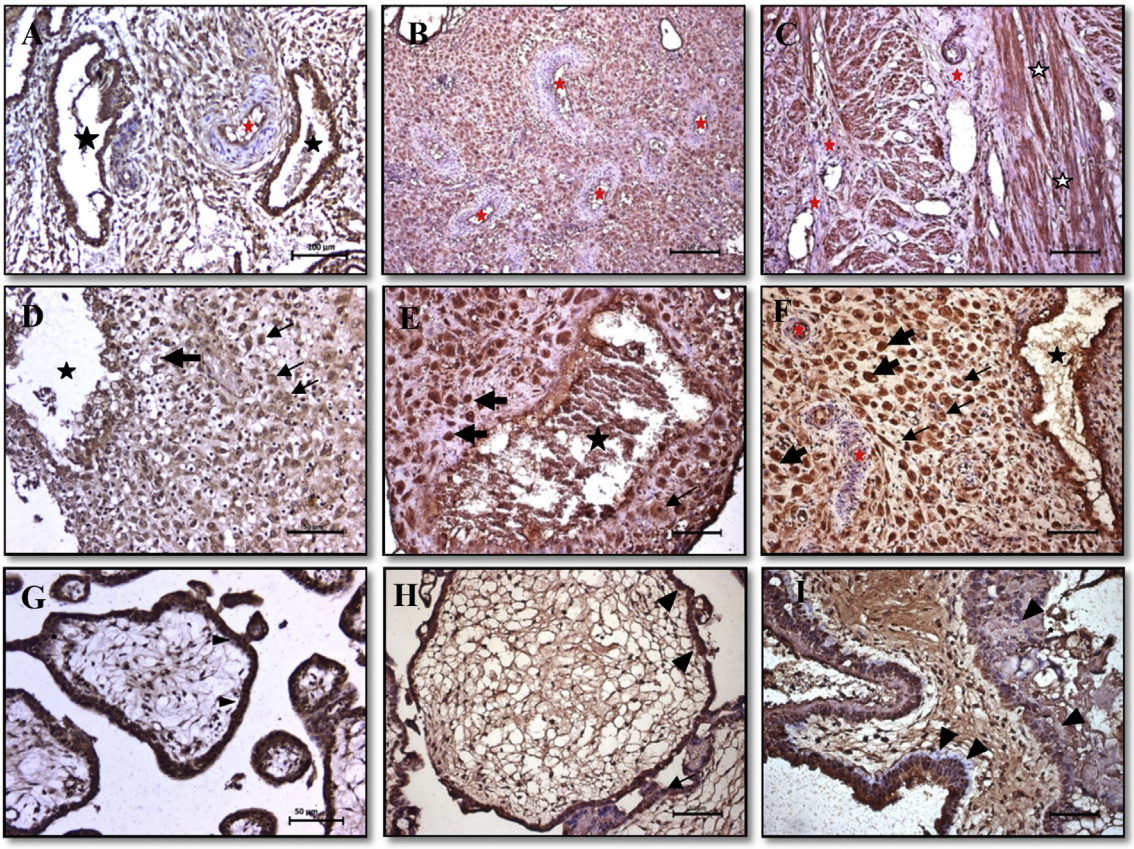

Fig. 1. Localization of LIF in normal first trimester (A,D,G), PHM (B,E,H) and CHM (C,F,I) placentas. A photomicrograph of LIF immunoperoxidase staining showing: A,B,C: Endometrial tissue. In all three groups, endothelial cells lining decidual blood vessels stained positive for LIF whereas smooth muscle cells in vessel wall did not. However, in CHM, myometrial smooth muscle cells were positive for LIF. black asterisk: immunoreactive endometrial gland, red asterisks: non reactive arterial smooth muscle cells, white asterisks: immunoreactive smooth muscle cells in myometrial smooth muscle. D,E,F: Decidualized endometrium. LIF expression was detected on endometrial glands of normal placentas, PHMs and CHMs. In normal placentas decidual cells stained positive for LIF. The decidual staining intensity decreased in PHM and CHM. Although decidual LIF staining was globally less intense in CHM group, staining intensity of decidual cells differed from each other. In normal placentas, extravillous trophoblasts showed faint LIF immunoreactivity. Strong LIF expression was detected on extravillous trophoblasts of PHM and CHM. long black arrows: decidual cells, thick black arrows: extravillous trophoblasts. G,H,I: Tertiary chorionic villi. In normal placentas and PHM, strong LIF immunostaining was detected in villous trophoblasts. In the case of CHM, weak immunostaining was observed in proliferating trophoblasts and no immunoreactivity was detected in cytotrophoblasts. black arrowheads: cytotrophoblasts. (A,B,C: ABC Method-Hematoxylin X100; D,E,F,G,H,I: ABC Method-Hematoxylin X200).

(Fig. 1D). In some EVT cells there was no immunoreactivity at all. In PHM group, EVTs showed stronger LIF expression (Fig. 1E). The strongest LIF expression was present in CHM group (Fig. 1F). EVT staining for LIF was significantly stronger in CHMs compared to normal first trimester placentas ($p < 0.016$).

In normal placentas, strong LIF immunostaining was observed in the cytoplasms of villous trophoblasts (VT), syncytiotrophoblasts and mesenchymal cells (Fig. 1G). Staining intensity of trophoblasts was similar to that seen in endometrial glands. A similar pattern of strong LIF immunoreactivity was seen in PHM group (Fig. 1H). On the contrary, LIF staining in hydropic chorionic villi of CHMs was globally less intense than in normal placentas. Faint immunostaining was observed in proliferating trophoblasts and no immunoreactivity was detected in cytotrophoblasts (Fig. 1I). A significantly lower level of LIF expression was present in VTs of CHM group compared to control ($p < 0.016$) and PHM ($p < 0.016$) groups.

Similar to LIF, IGF-1 immunoreactivity was localized in cytoplasm of glandular and decidual cells, stromal cells and EVTs in normal placentas (Fig. 2, A, D, G). Staining was much more intense in endometrial glands than in decidual and stromal cells. In the case of PHMs, IGF-1 immunostaining was localized in the cytoplasm of glandular cells and the staining was similar to that seen in normal placentas. In CHM group, glandular cells showed very weak cytoplasmic staining for IGF-1 (Fig. 2C). Glandular IGF-1 expression was significantly weaker in CHM group in comparison to control group ($p < 0.016$).

In normal placentas, weak IGF-1 expression was observed in decidual cells and the staining was similar in CHMs (Fig. 2, A, C, I). However decidual cells in PHMs showed stronger cytoplasmic staining for IGF-1 (Fig. 2B). Although statistical significance was found between groups ($p < 0.05$), no significant difference was detected after post-hoc analysis.

In all three groups, IGF-1 was also expressed in endothelial and smooth muscle cells of decidual blood vessels (Fig. 2 B, G, I). Endothelial IGF-1 staining was significantly weaker in complete moles compared to normal placentas ($p < 0.016$).

In normal first trimester placentas, faint cytoplasmic IGF-1 immunoreactivity was observed in EVT cells. In CHM group, IGF-1 expression was seen with the same localization and intensity as in normal placentas (Fig. 2I). While EVTs weakly expressed IGF-1 in normal and complete molar placentas, their staining was stronger in PHMs (Fig. 2B). Although statistical analysis showed difference between groups ($p < 0.05$), no statistical significance was found after post-hoc correction was performed.

In first-trimester villi, strong cytoplasmic IGF-1 staining was seen in cytotrophoblasts, syncytiotrophoblasts and mesenchymal cells (Fig. 2D). In contrast, chorionic villi in PHM and CHM groups showed less intense staining than in normal placentas (Fig. 2E and F). In the case of CHMs, faint immunostaining was observed in VTs and no

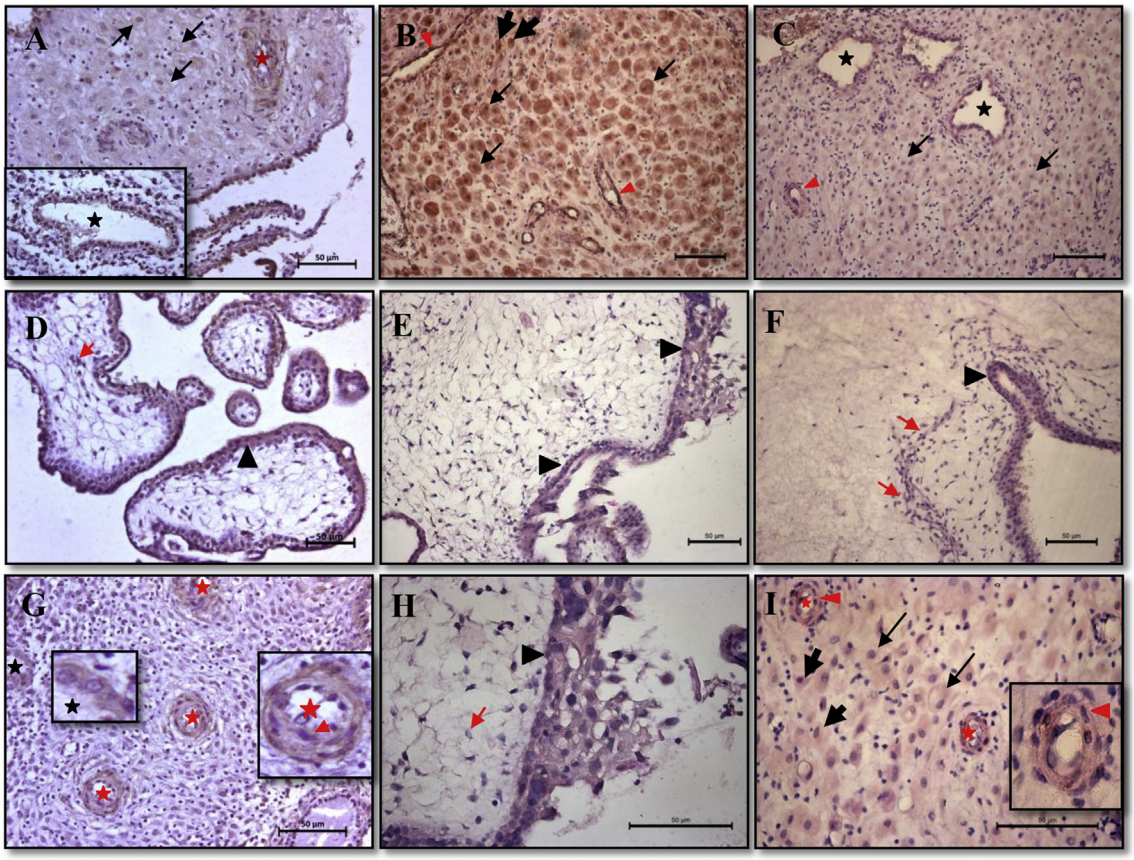

Fig. 2. Localization of IGF-1 in normal first trimester (A,D,G), PHM (B,E,H) and CHM (C,F,I) placentas. A photomicrograph of IGF-1 immunoperoxidase staining showing: A,B,C: Decidualized endometrial tissue. In normal placentas, strong IGF-1 was detected on endometrial glands and blood vessels while decidual cells showed weak staining. In PHM, IGF-1 was expressed strongly on decidual cells, extravillous trophoblasts and endothelial cells. In CHM, weak IGF-1 expression was detected on endometrial glands, decidual cells and vascular endothelium. black asterisk: endometrial gland, red asterisk: blood vessel, red arrowheads: endothelial cells, long black arrows: decidual cells, thick black arrows: extravillous trophoblasts. D,E,F: Tertiary chorionic villi. During normal first trimester development, villous trophoblasts and mesenchymal cells in chorionic villi showed strong IGF-1 expression. In PHM and CHM, IGF-1 staining became significantly weaker in cytotrophoblasts and mesenchymal cells. black arrowheads: cytotrophoblasts, red arrows: mesencyhmal cells. G: high magnification of the picture A showing glandular cells and vascular endothelia with strong immunoreactivity, H: High magnification of the picture E, tertiary chorionic villi, I: High magnification of the picture C showing vascular endothelium, decidual cells and extravillous trophoblasts with faint immunoreactivity. (A,B,C,D,E,F,G: ABC Method-Hematoxylin X200; H,I: ABC Method-Hematoxylin X400).

immunoreactivity was detected in mesenchymal cells (Fig. 2, F and H). A significantly lower level of IGF-1 expression was present in VTs of PHM ($p < 0.016$) and CHM ($p < 0.016$) groups compared to control group.

4. Discussion

Aberrant trophoblast proliferation is the hallmark of HM. In contrast proliferation and invasion of normal trophoblasts into decidua is a strictly controlled process. LIF and IGF-1 are two of the most important cytokines that are involved in the control of regular trophoblast proliferation and invasion. This study provides evidence that placental LIF and IGF-1 expressions are altered in molar pregnancies and suggests that LIF and IGF-1 may have a role in regulation of trophoblasts in HM.

LIF has been proposed to be playing an important role during placentation. LIF is known to regulate trophoblast function and promotes its proliferation and invasion into the decidua [18,19]. Our immunohistochemical analysis indicated that in normal pregnancies strong LIF expression is present in glandular and decidual cells demonstrating that decidua is an important source for LIF. Previous studies have also shown LIF secretion from both decidua and chorionic villi with 5- to 10-fold higher levels from decidua [20,21]. In PHM and CHM, glandular LIF expression was similar with normal pregnancies suggesting LIF expression in endometrial glands is not a part of decidual alterations in molar pregnancies. Indeed, LIF production from glandular cells was shown to be highly constitutive and relatively less regulated [22]. However LIF expression in decidual cells was much lower in CHMs compared to normal placentas. Decidua controls proliferation and invasion of trophoblasts. Various studies have shown that decidual cells actively produce LIF [19,22–24]. Moreover trophoblasts have LIF receptors and so trophoblasts are controllable [18,20]. LIF production by decidua may be a control mechanism of decidua to regulate trophoblast behavior. In CHMs where aberrant trophoblast proliferation takes place, decreased decidual LIF expression might be a component of the local response created in complete mole decidua. Indeed it has been suggested that regulation of decidual LIF expression may have an important role in the physiological and pathological processes in human implantation [22,24].

Several studies indicated that both VTs and EVTs express LIF and its receptor throughout pregnancy [8,20,23]. However, LIF expression of trophoblasts in HM remains to be clarified. In agreement with previous studies, our analysis showed that in normal first trimester pregnancies EVTs express LIF although very weakly. On the other hand, strong LIF expression was present in EVTs of molar pregnancies. LIF promotes adhesion and invasion of EVTs to the decidua [18]. It is possible that strong LIF expression found in molar EVTs might influence trophoblast behavior in CHMs. However further studies are needed to demonstrate LIF-molar trophoblast relationship. Although not studied in HM, LIF

was found to increase proliferation and invasiveness of choriocarcinoma cells suggesting a connection between LIF and proliferation and invasiveness of trophoblasts in choriocarcinoma [10]. In addition, human choriocarcinoma cells increase induction of HLA-G promoter, a molecule suggested to be involved in facilitating the immune tolerance of the conceptus, in response to LIF [25].

It is conceivable to assume that growth of chorionic villi is dependent on differentiation of cytotrophoblasts to syncytiotrophoblasts. LIF enhances differentiation of cytotrophoblasts to synctiotrophoblasts in a dose dependent manner [26]. Importantly, we found strong LIF expression in the chorionic villi of normal first trimester placentas, both in VTs and mesenchymal cells. In contrast, molar VTs showed weak LIF expression in CHM where disorganized villus structure was present. A previous study also found LIF and intense LIF receptor staining in normal chorionic villi which was mostly localized to trophoblasts and concluded that LIF action may be predominantly on trophoblasts through binding to LIF receptor [23]. It is also noteworthy that in LIF receptor mutant mice, placentas were found to be edematous, with large fluid-filled spaces, and lack organization into distinct spongio and labyrinthine trophoblast zone [27]. Based on these data, it seems reasonable to suggest that LIF is important for normal chorionic villi. However further study will be needed to identify the implications of decreased LIF expression in molar chorionic villi.

Presence of IGF-I at the maternal-fetal interface has been shown in several species and humans [9,28,29]. IGF-1 is produced by both the decidua and the chorionic villi and assumed to regulate placental development and function [29]. However little is known about IGF-1 role in HMs. Wihman et al. measured tissue and serum IGF-1 levels in samples from patients with HMs and normal placentas and found that tissue and serum total IGF-1 levels were significantly lower in molar pregnancies [30]. They suggested that the regulation of IGF-1 expression is on the transcriptional level and this finding may be reflecting a decreased production of the placental growth hormone variant in molar tissue; but they did not evaluate IGF-1 expression among placental cell types. In our study, we observed a similar low level of decidual cell IGF-1 expression in both normal and molar placentas. However IGF-1 expression in endometrial glands was significantly decreased in CHMs compared to normal first trimester placentas. Furthermore endothelial cells lining decidual blood vessels had significantly lower level of IGF-1 expression in complete moles. Taken together these findings suggest that regulation of endometrial IGF-1 expression during trophoblastic invasion through decidua in complete moles is different from that in normal pregnancies. Indeed, several studies have proposed that decidua may be using counter regulatory mechanisms on IGF-1 expression in response to different pathological situations [31–33]. An immunohistochemical study performed on placentas of intrauterine growth retarded fetuses showed increased decidual IGF-1 expression and concluded that decidua may be increasing IGF-1 expression as a positive feedback mechanism to restore normal growth [32]. Similarly, increased levels of decidual IGF-1 expression were reported in preeclampsia by the same authors [33].

Extensive research suggested potential role of genomic imprinting in HMs. IGF-2 gene is one of the genes identified to be paternally imprinted in human and it has been specifically examined in molar pregnancies [34]. A previous study demonstrated high level of IGF-2 expression in EVTs and cytotrophoblasts of CHM and high expression of IGF-2 in molar tissue was linked to hyperplastic phenotype [35]. In addition, it has been suggested that overexpression of paternally transcribed growth factors such as IGF2 could contribute to the malignant potential of CHM [36]. Similar to IGF-2, IGF-1 and LIF can enhance invasion and proliferation of trophoblasts. Furthermore IGF-1 was shown to enhance trophoblast survival [8,11,13,37]. Both LIF and IGF-1 genes have no imprinting effects shown to date. Whether differential expression of IGF-1 and LIF in molar pregnancies is related to genomic imprinting requires further investigation.

The differentiation of villous cytotrophoblasts into syncytiotrophoblast and EVTs is crucial for normal placental development. In agreement with previous studies, we observed strong IGF-1 expression in villous cytotrophoblasts, syncytiotrophoblasts and mesenchymal cells of normal placentas indicating that both VTs and mesenchyme are important sources of IGF-1 [31,38]. IGF-I is known to regulate the differentiation of cytotrophoblasts both into EVTs and syncytiotrophoblasts and because syncytiotrophoblasts are not capable of transcription, the syncytiotrophoblast layer is dependent on the continual proliferation and differentiation of cytotrophoblasts [14,15,39]. Therefore, it is clear that IGF-1 has a role in the processes that regulate placental cell turnover and trophoblast function. Our analysis showed weak IGF-I expression in chorionic villi of both partial and complete hydatidiform moles and no IGF-1 expression was detected in mesenchymal cells. This result was in agreement with the prior study by Wihman et al. which found lower levels of both IGF-1 mRNA and IGF-1 protein in hydatidiform moles [30]. It is possible that weak expression of IGF-1 in molar chorionic villi might influence regulation of trophoblasts.

In conclusion, this study has shown decreased expression of LIF and IGF-1 in complete mole decidua including decreases in glandular and endothelial IGF-1 expression and decidual cell LIF expression. It has also shown that in complete moles, LIF expression of molar trophoblasts during proliferation and invasion through decidua is different from normal first trimester trophoblasts. Finally, while normal chorionic villi showed strong expression of LIF and IGF-1, both LIF and IGF-1 expressions significantly decreased in chorionic villi of CHMs.

Our results are promising and encourage further studies in the field. Considering that LIF and IGF-1 are involved in regulation of trophoblast proliferation and invasion [8,11], differential expression of LIF and IGF-1 in molar trophoblasts and chorionic villi might have a role in regulation of trophoblasts in complete moles. In addition, decreased expression of glandular IGF-1 and decidual LIF may be related to the decidual changes during trophoblastic proliferation and invasion of decidua in CHMs. However the relationship between LIF and IGF-1 expressions and molar trophoblasts requires further investigations.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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References

[1] A.E. Szulman, U. Surti, The syndromes of hydatidiform mole. I. Cytogenetic and morphologic correlations, Am. J. Obstet. Gynecol. 131 (6) (1978) 665–671.
[2] P.A. Jacobs, C.M. Wilson, J.A. Sprenkle, N.B. Rosenshein, B.R. Migeon, Mechanism of origin of complete hydatidiform moles, Nature 286 (5774) (1980) 714–716.
[3] K. Yamashita, N. Wake, T. Araki, K. Ichinoe, K. Makoto, Human lymphocyte antigen expression in hydatidiform mole: androgenesis following fertilization by a haploid sperm, Am. J. Obstet. Gynecol. 135 (5) (1979) 597–600.
[4] F.J. Paradinas, R.A. Fisher, P. Browne, E.S. Newlands, Diploid hydatidiform moles with fetal red blood cells in molar villi. 1–Pathology, incidence, and prognosis, J. Pathol. 181 (2) (1997) 183–188.
[5] E.J. Watson, E. Hernandez, K. Miyazawa, Partial hydatidiform moles: a review, Obstet. Gynecol. Surv. 42 (9) (1987) 540–544.
[6] E. Menkhorst, A. Winship, M. Van Sinderen, E. Dimitriadis, Human extravillous trophoblast invasion: intrinsic and extrinsic regulation, Reprod. Fertil. Dev. 28 (4) (2016) 406–415.
[7] E. Dimitriadis, G. Nie, N.J. Hannan, P. Paiva, L.A. Salamonsen, Local regulation of implantation at the human fetal-maternal interface, Int. J. Dev. Biol. 54 (2–3) (2010) 313–322.

[8] P. Suman, S.S. Malhotra, S.K. Gupta, LIF-STAT signaling and trophoblast biology, JAK-STAT 2 (4) (2013) e25155.
[9] V.K. Han, N. Bassett, J. Walton, J.R. Challis, The expression of insulin-like growth factor (IGF) and IGF-binding protein (IGFBP) genes in the human placenta and membranes: evidence for IGF-IGFBP interactions at the feto-maternal interface, J. Clin. Endocrinol. Metabol. 81 (7) (1996) 2680–2693.
[10] J.S. Fitzgerald, S.A. Tsareva, T.G. Poehlmann, L. Berod, A. Meissner, F.M. Corvinus, B. Wiederanders, E. Pfitzner, U.R. Markert, K. Friedrich, Leukemia inhibitory factor triggers activation of signal transducer and activator of transcription 3, proliferation, invasiveness, and altered protease expression in choriocarcinoma cells, Int. J. Biochem. Cell Biol. 37 (11) (2005) 2284–2296.
[11] H. Lacey, T. Haigh, M. Westwood, J.D. Aplin, Mesenchymally-derived insulin-like growth factor 1 provides a paracrine stimulus for trophoblast migration, BMC Developmental Biology 2 (2002) 5.
[12] A.G. Miller, J.D. Aplin, M. Westwood, Adenovirally mediated expression of insulin-like growth factors enhances the function of first trimester placental fibroblasts, J. Clin. Endocrinol. Metabol. 90 (1) (2005) 379–385.
[13] G.S. Hamilton, J.J. Lysiak, V.K. Han, P.K. Lala, Autocrine-paracrine regulation of human trophoblast invasiveness by insulin-like growth factor (IGF)-II and IGF-binding protein (IGFBP)-1, Exp. Cell Res. 244 (1) (1998) 147–156.
[14] L.A. Milio, J. Hu, G.C. Douglas, Binding of insulin-like growth factor I to human trophoblast cells during differentiation in vitro, Placenta 15 (6) (1994) 641–651.
[15] J.D. Aplin, H. Lacey, T. Haigh, C.J. Jones, C.P. Chen, M. Westwood, Growth factor-extracellular matrix synergy in the control of trophoblast invasion, Biochem. Soc. Trans. 28 (2) (2000) 199–202.
[16] N. Ishikawa, Y. Harada, Y. Tokuyasu, M. Nagasaki, R. Maruyama, Re-evaluation of the histological criteria for complete hydatidiform mole: comparison with the immunohistochemical diagnosis using p57KIP2 and CD34, Biomed. Res. 30 (3) (2009) 141–147.
[17] H.D. Tsai, C.C. Chang, Y.Y. Hsieh, H.Y. Lo, Leukemia inhibitory factor expression in different endometrial locations between fertile and infertile women throughout different menstrual phases, J. Assist. Reprod. Genet. 17 (8) (2000) 415–418.
[18] J.S. Fitzgerald, T.G. Poehlmann, E. Schleussner, U.R. Markert, Trophoblast invasion: the role of intracellular cytokine signalling via signal transducer and activator of transcription 3 (STAT3), Hum. Reprod. Update 14 (4) (2008) 335–344.
[19] E.B. Cullinan, S.J. Abbondanzo, P.S. Anderson, J.W. Pollard, B.A. Lessey, C.L. Stewart, Leukemia inhibitory factor (LIF) and LIF receptor expression in human endometrium suggests a potential autocrine/paracrine function in regulating embryo implantation, Proc. Natl. Acad. Sci. U.S.A. 93 (7) (1996) 3115–3120.
[20] A.M. Sharkey, A. King, D.E. Clark, T.D. Burrows, P.P. Jokhi, D.S. Charnock-Jones, Y.W. Loke, S.K. Smith, Localization of leukemia inhibitory factor and its receptor in human placenta throughout pregnancy, Biol. Reprod. 60 (2) (1999) 355–364.
[21] K. Sawai, N. Matsuzaki, T. Kameda, K. Hashimoto, T. Okada, K. Shimoya, T. Nobunaga, T. Taga, T. Kishimoto, F. Saji, Leukemia inhibitory factor produced at the fetomaternal interface stimulates chorionic gonadotropin production: its possible implication during pregnancy, including implantation period, J. Clin. Endocrinol. Metabol. 80 (4) (1995) 1449–1456.
[22] A. Arici, O. Engin, E. Attar, D.L. Olive, Modulation of leukemia inhibitory factor gene expression and protein biosynthesis in human endometrium, J. Clin. Endocrinol. Metabol. 80 (6) (1995) 1908–1915.
[23] H.F. Chen, K.H. Chao, J.Y. Shew, Y.S. Yang, H.N. Ho, Expression of leukemia inhibitory factor and its receptor is not altered in the decidua and chorionic villi of human anembryonic pregnancy, Hum. Reprod. 19 (7) (2004) 1647–1654.
[24] K. Sawai, N. Matsuzaki, T. Okada, K. Shimoya, M. Koyama, C. Azuma, F. Saji, Y. Murata, Human decidual cell biosynthesis of leukemia inhibitory factor: regulation by decidual cytokines and steroid hormones, Biol. Reprod. 56 (5) (1997) 1274–1280.
[25] A.M. Bamberger, S. Jenatschke, H.M. Schulte, T. Loning, M.C. Bamberger, Leukemia inhibitory factor (LIF) stimulates the human HLA-G promoter in JEG3 choriocarcinoma cells, J. Clin. Endocrinol. Metabol. 85 (10) (2000) 3932–3936.
[26] K. Sawai, C. Azuma, M. Koyama, S. Ito, K. Hashimoto, T. Kimura, Y. Samejima, T. Nobunaga, F. Saji, Leukemia inhibitory factor (LIF) enhances trophoblast differentiation mediated by human chorionic gonadotropin (hCG), Biochem. Biophys. Res. Commun. 211 (1) (1995) 137–143.
[27] C.B. Ware, M.C. Horowitz, B.R. Renshaw, J.S. Hunt, D. Liggitt, S.A. Koblar, B.C. Gliniak, H.J. McKenna, T. Papayannopoulou, B. Thoma, et al., Targeted disruption of the low-affinity leukemia inhibitory factor receptor gene causes placental, skeletal, neural and metabolic defects and results in perinatal death, Development 121 (5) (1995) 1283–1299.
[28] B. de Vrijer, M.L. Davidsen, R.B. Wilkening, R.V. Anthony, T.R. Regnault, Altered placental and fetal expression of IGFs and IGF-binding proteins associated with intrauterine growth restriction in fetal sheep during early and mid-pregnancy, Pediatr. Res. 60 (5) (2006) 507–512.
[29] D.J. Hill, D.R. Clemmons, S.C. Riley, N. Bassett, J.R. Challis, Immunohistochemical localization of insulin-like growth factors (IGFs) and IGF binding proteins -1, -2 and -3 in human placenta and fetal membranes, Placenta 14 (1) (1993) 1–12.
[30] I.L. Wihman, K. Carlstrom, M. Faxen, Insulin-like growth factor-I in women with hydatidiform mole and in normal pregnancy, Obstet. Gynecol. 92 (3) (1998) 431–434.
[31] S. Sheikh, P. Satoskar, D. Bhartiya, Expression of insulin-like growth factor-I and placental growth hormone mRNA in placentae: a comparison between normal and intrauterine growth retardation pregnancies, Mol. Hum. Reprod. 7 (3) (2001) 287–292.
[32] H. Dalcik, M. Yardimoglu, B. Vural, C. Dalcik, S. Filiz, S. Gonca, S. Kokturk, S. Ceylan, Expression of insulin-like growth factor in the placenta of intrauterine growth-retarded human fetuses, Acta Histochemica 103 (2) (2001) 195–207.
[33] S. Ozkan, B. Vural, S. Filiz, P. Costur, H. Dalcik, Placental expression of insulin-like growth factor-I, fibroblast growth factor-basic, and neural cell adhesion molecule in preeclampsia, J. Maternal-fetal Neonatal Med. Offic. J. European Assoc. Perinatal Med. Fed. Asia Oceania Perinatal Societies Int. Soc. Perinatal Obstet 21 (11) (2008) 831–838.
[34] H.W. Li, S.W. Tsao, A.N. Cheung, Current understandings of the molecular genetics of gestational trophoblastic diseases, Placenta 23 (1) (2002) 20–31.
[35] C. Walsh, S.J. Miller, F. Flam, R.A. Fisher, R. Ohlsson, Paternally derived H19 is differentially expressed in malignant and nonmalignant trophoblast, Canc. Res. 55 (5) (1995) 1111–1116.
[36] R.A. Fisher, M.D. Hodges, Genomic imprinting in gestational trophoblastic disease–a review, Placenta 24 (Suppl A) (2003) S111–S118.
[37] S. Smith, R. Francis, L. Guilbert, P.N. Baker, Growth factor rescue of cytokine mediated trophoblast apoptosis, Placenta 23 (4) (2002) 322–330.
[38] C.Y. Wang, M. Daimon, S.J. Shen, G.L. Engelmann, J. Ilan, Insulin-like growth factor-I messenger ribonucleic acid in the developing human placenta and in term placenta of diabetics, Mol. Endocrinol. 2 (3) (1988) 217–229.
[39] K. Forbes, M. Westwood, The IGF axis and placental function. a mini review, Horm. Res. 69 (3) (2008) 129–137.